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ABI PRISM® 377 DNA Sequencer

XL Upgrade

User's Manual

Applied Biosystems

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ABI PRISM 377 XL Upgrade Overview

About This Manual

This manual describes the ABI PRISM® 377 DNA Sequencer enhancements included in the XL upgrade. Be sure to place this manual in your ABI PRISM 377 *DNA Sequencer User's Manual*.

Upgrade Overview

With the XL upgrade, the primary enhancement to the ABI PRISM 377 DNA Sequencer is the increase in the number of samples that can be analyzed simultaneously. This increased throughput was made possible by re-engineering the instrument and software to acquire more data as the laser scans across the gel.

XL-Specific Combs

This upgrade also includes new combs specifically designed for upgraded instruments. These combs are 48- and 64-well shark's-tooth and 50- and 66-well square-tooth combs. Other ABI PRISM 377 combs may be used.

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Safety Information

Site Preparation and Safety Guide

The *ABI PRISM 377 Site Preparation and Safety Guide* includes important safety information that should be read by all users before they operate the instrument. Topics covered in the guide include:

- An explanation of the safety labels affixed to the instrument
- Laser safety, including hazards and safe-operation requirements
- Chemical safety, including a waste profile and Material Data Safety Sheets for the chemicals commonly used with the instrument

We strongly recommend that this guide be kept readily available for reference at all times.

Ergonomic Hazard

! WARNING ! ERGONOMIC HAZARD. Performing loading activities may increase the risk of developing cumulative trauma disorders (repetitive motion or repetitive strain injuries), which include but are not limited to: tendonitis, tenosynovitis, epicondylitis, strains, and/or sprains. To reduce the risk of these disorders, the following recommendations have been developed to decrease awkward posture, repetitive motion, excessive force, static muscle loading, and soft tissue contact.

- Use an automated multi-channel pipet loader.
- Locate the instrument on a variable or predetermined-height worktable or lab bench.
- Use a stable stool or stepladder.
- Install adequate lighting in the appropriate area to facilitate loading.
- Ensure adequate front access to the instrument while performing loading activities.

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Loading Volumes

Loading References

The new combs require smaller loading volumes. For more information, refer to:

- The loading volume tables in this manual
- The ABI PRISM 377 *DNA Sequencing GeneScan Chemistry Guide* (P/N 4303188)
- The *Automated DNA Sequencing Chemistry Guide* (P/N 4305080)

Tips on Loading Small Volumes

Loading the small volumes required by the ABI PRISM 377 XL upgrade is easier if you use a gel-loading syringe, such as a multiplexed loader of four or eight syringes arrayed to match the XL upgrade well spacing. An alternative is an adjustable volume pipet with 0.17-mm gel loading tips. Volumes below 1.0 µL may be difficult to load with a pipet.

For best resolution, loading volumes should be smaller when using the 12-cm separation distance. Loading volumes of 0.5 µL are acceptable. You may find it possible and advantageous to use even smaller volumes.

Loading Volumes for Sequencing

Use the following table to determine resuspension and loading volumes for sequencing based on the comb configuration used.

	Comb Configuration (No. of Wells)		
	24/36 Wells	**48 Wells**	**64 Wells**
Resuspension vol.[a] (µL)	6–9	2–4	2–4
Loading vol. (µL)	1.5	0.5–1.0	0.5–1.0

a. 5:1 Deionized formamide to 50 mg blue dextran/mL 25 mM EDTA

! WARNING ! CHEMICAL HAZARD. Formamide is a known teratogen. It can cause birth defects. Wash thoroughly after handling formamide. Wear appropriate protective eyewear, clothing, and gloves. Obtain a copy of the MSDS from the manufacturer. Wash thoroughly after handling formamide.

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Loading Volumes for GeneScan

Use the following table to determine component and loading volumes for GeneScan based on the comb configuration used.

	Comb Configuration (No. of Wells)		
	24/34 Wells	**50 Wells**	**66 Wells**
Deionized formamide (µL)	2.5	2.5	2.5
50 mg Blue dextran/mL 25 mM EDTA (P/N 402055) (µL)	0.5	0.5	0.5
Size standard[a] (µL)	0.5	0.5	0.5
DNA (µL)	1.5	1.5	1.5
Loading vol. (µL)	1.5	1.0–1.5	0.5–1.0

a. If the peak heights fall below 50 fluorescent units, increase the concentration by doubling the volume of the size standard in the cocktail.

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Lanes, Combs, and Run Modes

Lanes, Combs, and Run Modes Table

The number of lanes, the types of combs used, and the corresponding run modes are shown in the following table.

Lanes	Comb[a]	Run Mode
24	Shark's-tooth	Full Scan
24	Square-tooth	Full Scan
32	Shark's-tooth	Full Scan
34	Square-tooth	Full Scan
36	Shark's-tooth	Full Scan
36	Square-tooth	Full Scan
48	Shark's-tooth	XL Scan
50	Square-tooth	XL Scan
64	Shark's-tooth	XL Scan
66	Square-tooth	XL Scan

a. Well spacing for the XL combs is 9 mm on center to accommodate multichannel pipettes.

IMPORTANT For 48, 50, 64, and 66 lanes, you must use XL Scan Run mode.

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Silanizing Agent

Silanizing Agent for Preparing Gels

Purpose

When using a square-tooth comb, silate the unnotched glass plate in the well area to facilitate loading samples. Use a silanizing agent such as -methacryloxypropyltrimethoxysilane (Sigma P/N M6514) to bind the gel to the glass.

Procedure

Perform the following procedure every time a new gel is poured.

! WARNING ! CHEMICAL HAZARD. Silanizing agents are irritating to the eyes, respiratory system, and skin. Always work in a fume hood. Obtain a copy of the MSDS from the manufacturer. Wear appropriate protective eyewear, clothing, and gloves.

To apply a silanizing agent and remove a gel after a run:

Step	Action
1	Wash the unnotched glass plate in detergent and distilled water. Rinse well in distilled water then air dry.
2	Apply a small amount of silanizing agent to a cotton swab and spread it across the top 3–5 cm of the unnotched glass plate.
3	Allow the agent to dry thoroughly.
4	Assemble the plates for the run as usual.
5	Remove the gel from the silated area with 0.1 N HCl or 0.1 N NaOH, or scrape off gently.

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Software

Collection Software

The current collection software is version 2.5.

GeneScan Analysis

The current GeneScan Analysis software is version 3.1 with GSGelTracker.

Sequence Analysis

The current Sequence Analysis software is version 3.3 with SAGelTracker.

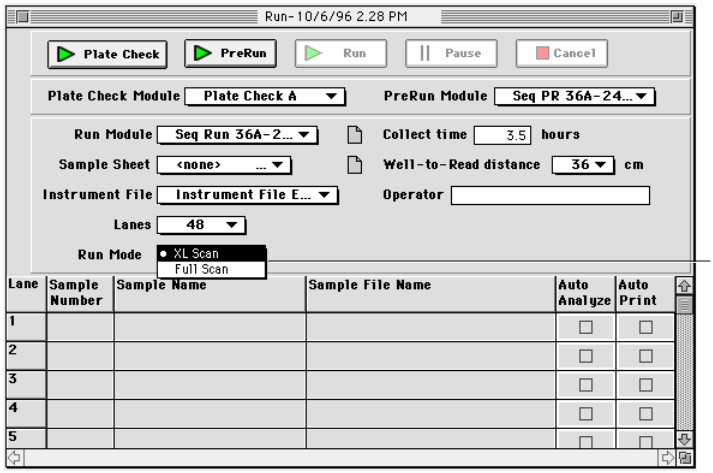
Preference File

Define default values in the new software following the procedures described under “Setting Preferences” in the *ABI PRISM* 377 DNA Sequencer *User Manual.*

The default values you define for Sequencing or GeneScan run modules and sample sheets are maintained in the Preference file.

Sequencing and GeneScan Run Windows

The Sequencing and GeneScan Run windows now include the Run Mode pulldown menu, as shown below. You can now choose either Full Scan (standard with the ABI PRISM 377) or XL Scan (with the upgrade). See “Lanes, Combs, and Run Modes Table” on page 5.



Run Mode pulldown menu

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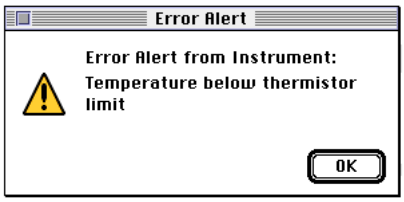
Error Alert Message

The following error message has been added to alert you of a possible problem with the heat-transfer plate thermisters.



This message may appear when you open data collection and start a plate check, a pre-run, or a run if one of the thermisters is not functioning.

If this message appears, schedule a service call to troubleshoot the thermister and continue to operate the instrument as usual.

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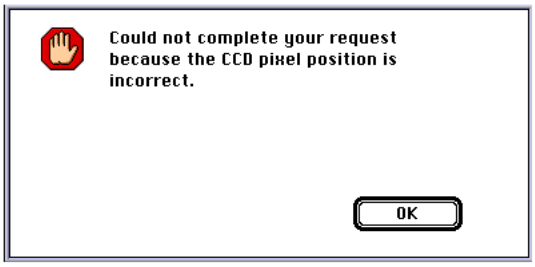
CCD Pixel Position Value

CCD Pixel Position Overview

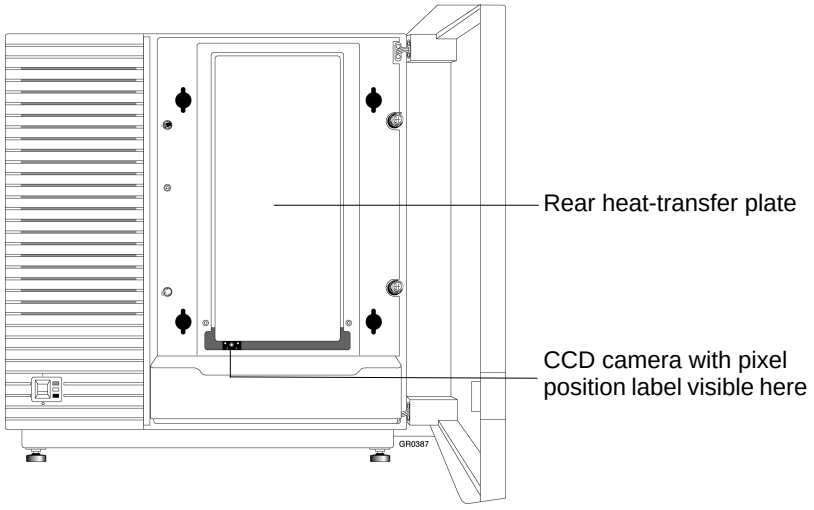
The instrument is shipped with the correct CCD pixel position value in memory. When a run is started, the software checks for a value greater than zero. If the value is lost from memory, an error message is displayed at the beginning of the run as shown below.



The CCD pixel position value may become corrupted as the result of a power surge or failure. If this occurs, you must find the correct value and enter it as described in the following procedures before starting a run.

Locating the CCD Pixel Position Label

The correct CCD pixel position value is written on a white label on the CCD camera. It is visible from the front through the opening below the rear heat-transfer plate, as shown below.



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Note The following procedure requires the use of a flashlight.

To locate the CCD pixel position value on the label:

Step	Action
1	Open the front door of the instrument.
2	Shine a light through the opening below the rear heat-transfer plate to view the white label on the CCD camera.
3	Record the value from the white label.
4	If you cannot find the label, call technical support.

Entering the Correct CCD Pixel Position Value

To enter the correct CCD pixel position value in the calibration file:

Step	Action
1	Turn power on to the instrument.
2	Select Manual Control from the Window menu.
3	Open the Fxn Name pulldown menu.
4	Select the CCD Pixel Position function. The current pixel position value is displayed.
5	Compare the displayed value with the value on the white label.

If the pixel position is...	Then...
the same as the value on the white label	Stop here.
different than the value on the white label	a. Select the text box and type the correct CCD pixel position value. b. Click Execute.

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Using Calibration File Make

IMPORTANT You must have the correct CCD pixel position value in instrument memory before using the Calibration File Make or Send functions.

Use the Calibration File Make function from the Manual Control Window to create the ABI 377XL Calibration file and store the CCD pixel position value and instrument serial number in it.

When created, the file is named ABI 377XL Calibration and is placed in the Preferences folder inside the System folder. To read, edit, or print the Calibration file, use the SimpleText application.

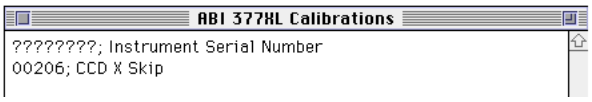
Creating a Calibration File

To create a Calibration file with Calibration File Make:

Step	Action
1	Turn on power to the instrument.
2	Select Manual Control from the Window menu.
3	Open the Fxn Name pulldown menu.
4	Select Calibration File Make.
5	Click Execute.

Entering the Serial Number

To enter the instrument serial number in the calibration file:

Step	Action
1	Make a note of the serial number from the back of the instrument.
2	Open the System Folder and the Preferences Folder inside the System Folder.
3	Open the ABI 377XL Calibration file.
4	Using SimpleText, type the instrument serial number or name (up to eight characters) by replacing the question marks. 
5	Select Save from the File menu.
6	Close the ABI 377XL Calibration File window.
7	Quit the SimpleText program.

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Using Calibration File Send

IMPORTANT You must have the correct CCD pixel position value in instrument memory before using the Calibration File Make or Send functions.

After creating a Calibration file, use Calibration File Send from the Manual Control window to send the instrument's serial number (or name) and CCD pixel position value to the instrument. These values will be displayed in the Collection Log window.

The serial number (or name) is transferred into all sample files created by the instrument. It can be viewed in the annotative view of the sample file. This is useful for identifying instruments, particularly if you operate more than one instrument.

To transfer a Calibration file with Calibration File Send:

Step	Action
1	Turn on power to the instrument.
2	Select Manual Control from the Window menu.
3	Open the Fxn Name pulldown.
4	Select Calibration File Send.
5	Click Execute.

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Module Files

Unused Module Folder

Move all of the module files that you do not intend to use from the Modules folder into the Unused Modules folder.

Collection 2.5 Module Files

The following table lists the module files in Collection Software v. 2.5.

2.5 Collection Module Files[a]

377XL Modules	Chiller Modules
GS PR 12A-1200	GS PR 12A-1200 CHILLER
GS PR 12A-2400	GS PR 12A-2400 CHILLER
GS PR 12C-1200	GS PR 12C-1200 CHILLER
GS PR 12C-2400	GS PR 12C-2400 CHILLER
GS PR 12D-1200	GS PR 12D-1200 CHILLER
GS PR 12D-2400	GS PR 12D-2400 CHILLER
GS PR 12F-1200	GS PR 12F-1200 CHILLER
GS PR 12F-2400	GS PR 12F-2400 CHILLER
GS PR 36A-1200	GS PR 36A-1200 CHILLER
GS PR 36A-2400	GS PR 36A-2400 CHILLER
GS PR 36C-1200	GS PR 36C-1200 CHILLER
GS PR 36C-2400	GS PR 36C-2400 CHILLER
GS PR 36D-1200	GS PR 36D-1200 CHILLER
GS PR 36D-2400	GS PR 36D-2400 CHILLER
GS PR 36F-1200	GS PR 36F-1200 CHILLER
GS PR 36F-2400	GS PR 36F-2400 CHILLER
GS Run 12A-1200	GS Run 12A-1200 CHILLER
GS Run 12A-2400	GS Run 12A-2400 CHILLER
GS Run 12C-1200	GS Run 12C-1200 CHILLER
GS Run 12C-2400	GS Run 12C-2400 CHILLER
GS Run 12D-1200	GS Run 12D-1200 CHILLER
GS Run 12D-2400	GS Run 12D-2400 CHILLER
GS Run 12F-1200	GS Run 12F-1200 CHILLER
GS Run 12F-2400	GS Run 12F-2400 CHILLER
	GS Run 2140V A CHILLER
	GS Run 2140V C CHILLER

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2.5 Collection Module Files[a] *(continued)*

377XL Modules	Chiller Modules
	GS Run 2140V D CHILLER
	GS Run 2140V F CHILLER
GS Run 36A-1200	GS Run 36A-1200 CHILLER
GS Run 36A-2400	GS Run 36A-2400 CHILLER
GS Run 36C-1200	GS Run 36C-1200 CHILLER
GS Run 36C-2400	GS Run 36C-2400 CHILLER
GS Run 36D-1200	GS Run 36D-1200 CHILLER
GS Run 36D-2400	GS Run 36D-2400 CHILLER
GS Run 36F-1200	GS Run 36F-1200 CHILLER
GS Run 36F-2400	GS Run 36F-2400 CHILLER
	GS Run 60W A CHILLER
	GS Run 60W C CHILLER
	GS Run 60W D CHILLER
	GS Run 60W F CHILLER
Plate Check A	Plate Check A CHILLER
Plate Check C	Plate Check C CHILLER
Plate Check D	Plate Check D CHILLER
Plate Check E	Plate Check E CHILLER
Plate Check F	Plate Check F CHILLER
Seq PR 36A-1200	Seq PR 36A-1200 CHILLER
Seq PR 36A-2400	Seq PR 36A-2400 CHILLER
Seq PR 36E-1200	Seq PR 36E-1200 CHILLER
Seq PR 36E-2400	Seq PR 36E-2400 CHILLER
Seq PR 48A-1200	Seq PR 48A-1200 CHILLER
Seq PR 48E-1200	Seq PR 48E-1200 CHILLER
Seq Run 36A-1200	Seq Run 36A-1200 CHILLER
Seq Run 36A-2400	Seq Run 36A-2400 CHILLER
Seq Run 36E-1200	Seq Run 36E-1200 CHILLER
Seq Run 36E-2400	Seq Run 36E-2400 CHILLER
Seq Run 48A-1200	Seq Run 48A-1200 CHILLER
Seq Run 48E-1200	Seq Run 48E-1200 CHILLER

a. PR = Prerun; Seq = Sequencing; GS = GeneScan

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Firmware

Position-Based Integration Scheme

Due to the increased demand for positional accuracy of the detection optics, a new integration scheme is used. Previously, a given time was given to each channel, before reading the CCD camera and switching to the next channel. This release of firmware uses position-based CCD integration, where predetermined stage positions determine when to switch.

Resetting the Instrument

Reset the instrument under the following situations.

Reset the instrument when...	To reset, perform a...	Resetting is necessary because...
new firmware version has been downloaded.	single reset. Press the reset button once.	the running TPU code needs to be reinitialized. **IMPORTANT** Failure to reset the instrument will cause a lockup.
the instrument does not respond to commands or it responds inappropriately.	total reset. Follow the procedure that follows.	the firmware image may be corrupted.

Performing a Total Reset

To perform a total reset:

Step	Action
1	Using the eraser end of a pencil or similar object, press the red reset button on the back of the instrument twice in rapid succession.
2	Quit the data collection software.

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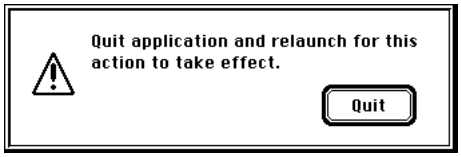
Step	Action
3	Launch the data collection software. The firmware is automatically downloaded to the instrument. This will take 60–90 seconds.
4	In response to this prompt, quit and then relaunch the data collection software. 
5	Check the CCD pixel position value as follows: a. Open the Window menu and select Manual Control. b. Open the Fxn Name menu and select CCD Pixel Position Value. The value is displayed in the Value box. If it matches the value on the instrument, it is OK. If it does not match, correct the value.

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